A MODIFICATION OF CHICK EMBRYO RESISTANCE TO VARIOUS INFECTIONS UNDER THE EFFECT OF POLYSACCHARIDES ISOLATED FROM NONPATHOGENIC MICROBES

Translation No. 1693

May 1966

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A MODIFICATION OF CHICK EMBRYO RESISTANCE TO VARIOUS INFECTIONS UNDER THE EFFECT OF POLYSACCHARIDES ISOLATED FROM NONPATHOGENIC MICROBES

[Following is the translation of an article by G. Ye. Vaysberg and T. V. Golosova, Central Institute for the improvement of Surgeons, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 6, 1964, pp 96-101. It was submitted on 21 Sep 1962. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Nonspecific resistance to infections and the possibility of increasing it with the help of polysaccharides of a bacterial origin are of considerable interest from the point of view of studying the nature of this phenomenon and the feasibility of the practical application of biologically active bacterial polysaccharides.

In previous works (1958, 1959, 1961) we demonstrated that the parenteral administration of polysaccharides, acetoxan and prodigiosin, which are isolated from nonpathogenic microorganisms, sharply increases the resistance of animals against a number of bacterial infections. The mechanism of the stimulating effect of these substances has been partially interpreted at the present time. Along with this, it is known that under specific conditions the resistance of embryos to bacterial and viral infections is very low. This peculiarity is made use of for the cultivation of certain microorganisms (rickettsiae, viruses) in the chick embryo. In connection with this, the problem arose at what stage of the embryonic life are the systems formed which take part in the nonspecific resistance of an animal to infections, and is it possible to activate them in an embryo with the help of bacterial polysaccharides which stimulate the nonspecific resistance of adult animals. For the purpose of studying these problems we investigated the influence of acetoxan, prodigiosin and glucan -- polysaccharides which were isolated from various nonpathogenic microbes -- on the resistance of chick embryos to bacterial infection.

The first group of tests was set up in chick embryos of various ages. Twenty-four hours prior to infection we introduced into the allantoic cavity of the embryos 0.2 ml of a solution of the polysaccharide being tested, or an equal volume of a finely ground suspension of a preparation, if the latter was not dissolved in water (acetoxan and glucan). An equal volume of physiological solution was administered to the control embryos in place of the polysaccharide. We used the following polysaccharides in these tests: Acetoxan B, isolated by us from Acetobacter Xylinum, Prodigiosin, isolated by us from B. prodigiosum (strain K), and glucan, isolated from baker's yeast and characterized as a pure polymer of glucose (Yermolyeva et al., 1961). After 24 hours following the administration of the polysaccharides being studied, the embryos were infected with a 24-hour agar culture of Staphylococcus aureus or pathogenic E. coli. Each 0.2 ml of microbial

suspension contained from 800--1000 microbial cells (based on the bacterial standard). The stated dose caused the death of 90--100% of the embryos of the control group by the 3--4th day following infection. As a rule the embryos were observed for 7 days. Each group usually consisted of 15 embryos.

Below we present the average data from several repeated tests, set up with embryos at the ages of 7, 9, 13 and 15 days (15, 15, 30 and 45 embryos correspondingly), which were infected with Staphylococcus aureus after 24 hours following the administration of 100 μg of acetoxan. The control embryos were infected following the administration of a physiological solution, or they received only a preparation for the purpose of checking the harmlessness of the latter. All told 100 embryos of the appropriate ages were used in the control investigations.

Figure 1 presents the results obtained, which are expressed by the index of survival of the embryos over a stretch of 7 days following infection. The survival indexwas the ratio of surviving embryos to the number of embryos in the test. It turned out that the survival rate of 7-day embryos was hardly any different from the survival rate of the control (index of survival 0), however the administration of acetoxan B somewhat slowed down the onset of death of the embryos. This was especially noticeable on the 1st and 2nd day following infection: In the control group on the 2nd day following infection only 20% survived, and in the test group -- 40% of the embryos. A great delay in the death of embryos from staphylococcal infection was noted in the group of 9-day embryos, which had received 100 μg of acetoxan B 24 hours prior to infection, though in this group all the embryos died by the end of the test..

Only following the 11th day of embryonic life the administration of acetoxan B was expressed not only in the delay of death, but also in an increase in the number of surviving embryos: By the end of the test 40% of the 13-day embryos were alive and 76% of the 15-day embryos. Normal chicks were hatched from the embryos which remained alive.

An analogous regularity is noted following the infection of embryos with pathogenic E. coli.

The data obtained testified that following the administration of a biologically active polysaccharide which stimulated the resistance of an organism to infection, mechanisms are activated which are lacking or weakly developed in the early stages of ontogenetic development (7- and 9-day embryos). As they developed further, adaptations emerged or were strengthened which gave them the capability to react to the administration of polysaccharide, and by the 15th day of embryonic life they, under the influence of a biologically active polysaccharide, guaranteed the complete survival of the largest segment of the embryos, while in the control group there was 100% death of the embryos.

It should be pointed out that according to the data of some investigators the reticulo-endothelium system is formed by the 12th day, and by the 16th day of embryonic life its phagocytic activity is well expressed. At

the same time there are indications that in the early stages of development the chick embryo does not contain γ-globulin, its content reaches a maximum by the 16th day of embryonic life (Green and Lorincz). Apparently these data can be viewed as the manifestation of the evolutionary nature of the development of mechanisms which guarantee the resistance of embryos to infection (Peres del Castillo, 1958; Finkelstein, 1961).

Since it was demonstrated earlier by us jointly with Braude that both acetoxan and prodigiosin and glucan stimulate the phagocytic activity of the cells of the reticulo-endothelial system, it can be proposed that the above exposed dependency of the increase of the resistance of embryos under the influence of polysaccharides on growth is tied in with the corresponding mechanisms of nonspecific resistance, in particular with the development of the phagocytic activity of the cells of the reticulo-endothelial system.

We set up further tests on 13-day embryos, which were sufficiently mature to react clearly to the administration of a biologically active polysaccharide. On the same model of staphylococcal infection of embryos a comparison was made of the effect of several biologically active polysaccharides, isolated from various microorganisms. In this group of tests we used 200 embryos, and 24 hours prior to infection each of them received either 5 μg of prodigiosin or 100 μg of acetoxan B or glucan.

As is seen from figure 2, all 3 of the polysaccharides tested clearly delayed the development of staphylococcal infection, and the survival rate of the test animals was sharply increased in comparison with the control. In the test groups no less than 40% survived, and with the administration of prodigiosin, the activity of which is significantly higher in very small doses, 60% of the embryos. Similar results were also obtained when the embryos were infected with pathogenic E. coli (strain 145) or Salmonella typhi. The data obtained showed that in principle the resistance of the embryos to infection is increased regardless of the nature of the causative agent. This supports the concept that the tested polysaccharides are stimulators of the nonspecific immunity of an embryo.

In order to clear up how rapidly it emerges and how stable is the condition of increased resistance to infection, caused by the administration of polysaccharide into the allantoic cavity of an embryo, four groups of embryos (all told 150 embryos) received prodigiosin (10 μg) in a period of 5, 3 and 1 days, and 6 and 3 hours prior to infection with a lethal dose of staphylococci. In the control group the embryos (all told 90) received an equal amount of physiological solution in the same periods.

As is seen from figure 3, the optimum results are obtained in that group of embryos in which the prodigiosin was administered 24 hours prior to infection. The highest survival rate is noted in this group (60% by the end of the test). At the same time a great delay in the development of infection was observed --on the 4th day of infection 90% of the embryos survived, while there was 100% death of the control group. Following the administration of polysaccharide 6 hours prior to infection the effect of the influence of prodigiosin was considerably lower, though a delay in the development of infection is expressed quite clearly; and with an increase of

this interval up to several days, the effectiveness of the administration of prodigiosin exceeded that which was noted following the short time interval (3--6 hours), but remained at a lower level than when administered in 24 hours. Analogous data is obtained when embryos are infected in the same periods with E. coli. The same regularity is noted in the tests with acetoxan B.

The stated data leads to the assumption that the reorganization of the organism under the influence of prodigiosin or acetoxan B, which is already noticeable in 3 hours following its administration, is not developed immediately and only gradually reaches the highest level, which is noted after 24 hours. After several days following the administration of prodigiosin its effectiveness is gradually lowered, though even after 5 days the level of resistance remained sufficiently high; the death of the embryos is noticeably slowed down, and by the end of the test (on the 7th day following infection and on the 12th day following the administration of prodigiosin) 40% of the embryos survived. These results testify to the significant stability of the resistance which is stimulated by the polysaccharides being tested. In this respect the reaction of the chick embryos is completely analogous to that observed during septic infections in mice (Yermolyeva et al., 1958, and others).

As was pointed out above, the data on the survival rate of embryos which had received prodigiosin simultaneously with infection with staphylococci, or after 3 and 6 hours and one day following infection, were compared with the survival rate of embryos which were infected after 24 hours following the administration of an equal dose of acetoxan or prodigiosin. As is seen from these tests (160 test embryos and 65 control), the administration of prodigiosin simultaneously with the infection, after 3--6 hours, or even after 24 hours following infection (see figure 3), noticeably delayed the development of infection and clearly raised the survival rate of the test embryos in comparison with the control. A similar result was obtained in the tests with acetoxan B.

Meanwhile, it is known from the data which was obtained earlier on white mice that the resistance of mice to infection is developed only after 4--6 hours following the administration of an effective dose of acetoxan A or acetoxan B, but in this period it still does not reach the highest level, which is noted after 20--24 hours following the injection of polysaccharide. Following the administration of prodigiosin, the analogous reorganization which is excited by it is developed somewhat more rapidly: A sharp increase in the resistance to infection is noted already after 5 hours following the administration of 10 μg of prodigiosin (the survival index of the test group of mice equaled 0.9 μg, while the index in the control group equaled 0.1 μg). However, with a 2-hour time interval between the administration of prodigiosin and the infection, the index of survival in the test group still did not differ from the index in the control. In embryos which have reached a sufficient level of development (beginning with the 11--13th day), the reaction, leading to a significant increase of resistance, is developed significantly more rapidly; already after 3 hours following the administration

of acetoxan or prodigiosin the resistance of the embryos turned out to be sufficiently high enough to guarantee the survival of 40% of the embryos.

Such a difference in the change of the course of infection in mice and embryos may apparently be explained by the difference in the condition of the stimulated mechanisms. Since in our tests the infectious process caused by us also led to the death of the control embryos and mice by the 3rd day following their infection, it is difficult to conjecture that the difference in the effect from the administration of prodigiosin is caused by a difference in the course of the infectious process in the embryo and the mouse. In connection with this, the assumption arises that in the chick embryo the mechanisms which are responsible for resistance to infection are reorganized more rapidly and with a greater intensity than in the mouse. Thanks to this, even with the administration of acetoxan and prodigiosin simultaneously or following infection, that is, when the infectious process has reached a significant development, 40% of the embryos turned out to be in a condition to completely suppress it.

Conclusions

1. Biologically active polysaccharides from nonpathogenic microorganisms -- acetoxan, prodigiosin and glucose -- are capable of stimulating the nonspecific resistance of embryos to certain infections.

2. In the early stages of development the chick embryos did not react to the administration of biologically active polysaccharides with a sufficient increase of resistance to a number of bacterial infections.

3. As a measure of the development of the embryos, this capability was intensified and was well expressed and comparatively stable in 13-day embryos, and in 15-day embryos it reached a still higher level. At this age the administration of polysaccharides changed the development of infection in the embryos not only when they were administered prophylactically, but also when administered simultaneously with infection or following infection. Additional investigations are required for clearing up this peculiarity.

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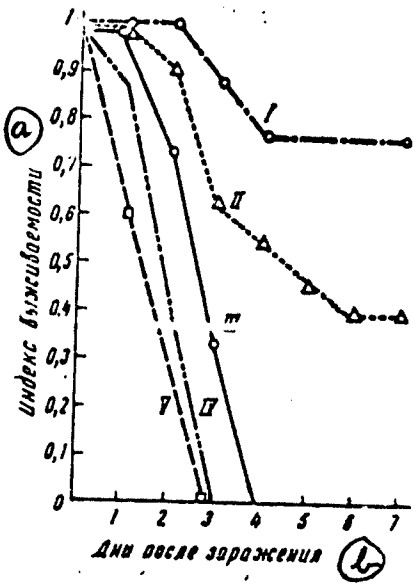

Figure 1. Influence of acetoxan B on the survival rate of embryos of a various age following staphylococcal infection.

I - 15-day embryos, II - 13-day embryos, III - 9-day embryos, IV - 7-day embryos, V - control.

a - survival index; b - days following infection.

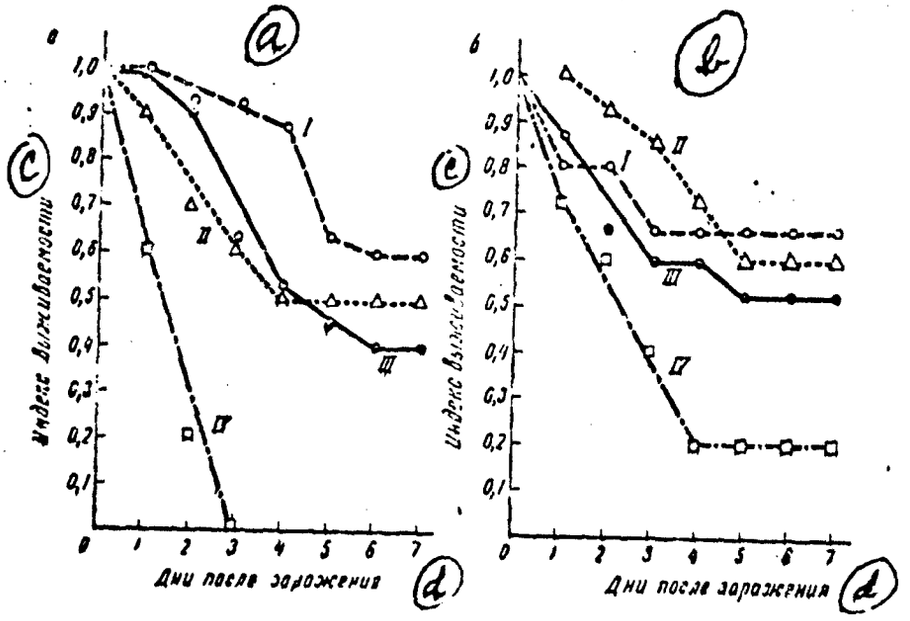

Figure 2. Influence of acetoxan B, prodigiosin and glucan on the survival rate of 13-day embryos, infected with staphylococci (a) and E. coli (b).

I - prodigiosin; II - acetoxan; III - glucan; IV - control.

c - survival index; d - days following infection.

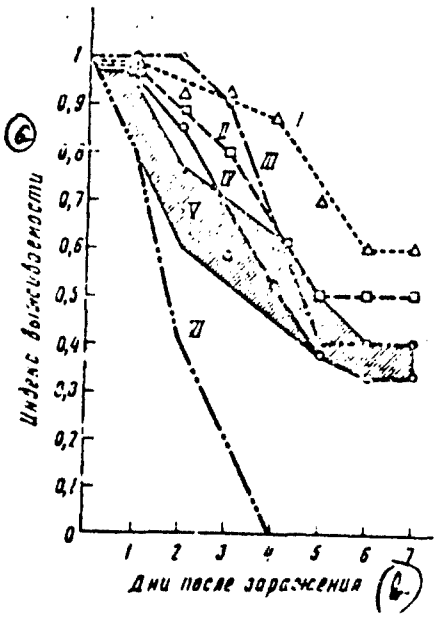

Figure 3. Dependency of the survival rate of embryos, infected with staphylococci, on the period of administration of prodigiozan.

I - prodigiozan administered 5 days prior to infection; II - prodigiosin administered 24 hours prior to infection; III - prodigiosin administered 3 days prior to infection; IV - prodigiosin administered 3--6 hours prior to infection; V - prodigiosin administered after 3, 6 and 24 hours and simultaneously with infection; VI - control.

a - survival index; b - days following infection.